PATENT
Our Docket: P-LA 1245

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Border and Ruoslahti	Examiner: E. Rabin
Serial No.: 08/349,479	Group Art Unit: 1644
Filed: December 2, 1994	
For: INHIBITING TRANSFORMING GROWTH FACTOR β TO PREVENT ACCUMULATION OF EXTRACELLULAR MATRIX	

#600

Assistant Commissioner of Patents
Washington, D.C. 20231

DECLARATION UNDER 37 C.F.R. § 1.131

Sir:

1. We, Erkki Ruoslahti and Wayne Border, are coinventors named on the above-identified patent application, which claims priority to United States application Serial No. 07/416,656 (hereinafter '656), originally filed on October 3, 1989.

2. We are both Medical Doctors and state herein under oath that we conceived the claimed invention in the United States of America prior to December 22, 1988, which is prior to the effective publication date of United States Patent 5,571,714 (hereinafter the '714 patent); prior to the publication date of Conner et al., J. Clin. Invest., **83**:3039-3045 (May 1989); and prior to the publication date of MacKay et al., J. Clin. Invest., **83**:1160-1167 (1989). We diligently

pursued, and conducted all experiments and work related to, the claimed invention in the United States.

3. As evidence that we conceived of the invention prior to December 22, 1988, we enclose as Exhibits A and B, photocopies of laboratory notebook pages. The dates of the notebook pages have been redacted, but are prior to December 22, 1988; except for the notebook page 3 of Exhibit A, which includes an "Animal Procedure Request" form that indicates the dates the animals were bled for anti-TGFβ serum as December 13, 16, and 21 of 1988.

4. Exhibit A contains three notebook pages, each page showing a protocol used, prior to December 22, 1988, in the development of a rabbit anti-TGF-β antiserum used in the invention. The protocol included the injection of linear and cyclic TGF-β peptides.

5. Exhibit B provides three laboratory notebook pages demonstrating experiments performed prior to December 22, 1988, the experiments designed to characterize the ability of TGF-β inhibitory agents to decrease the secretion, *i.e.*, accumulation, of extracellular matrix components, specifically, decorin and biglycan. A rat glomerular culture experimental model was used as described in the Specification of the '656 application, on pages 16 to 17, Example III(a), entitled "Induction of Experimental Glomerulonephritis;" and

pages 18 to 19, Example III(c), entitled "Glomerular Culture."

6. Page 1 of Exhibit B demonstrates the ability of anti-TGF-β antibody to inhibit the secretion of proteoglycans in the above-described rat glomerular culture model. After addition of the anti-TGF-β antibody to the glomerular cultures, basically as described in the Specification on pages 20 to 22, Example IV and pages 23 to 24, Example VII, extracellular matrix components were isolated and identified by polyacrylamide gel electrophoresis (SDS-PAGE). The glomerular cultures had been metabolically labeled with ^{35}S-methionine, as described in Example II(1) of the Specification, and the amounts of proteoglycans in the gels were analyzed by laser densitometry.

7. Page 2 of Exhibit B demonstrates the ability of RGD peptides to inhibit the secretion of proteoglycans in a rat glomerular cell culture model. This experiment was designed essentially the same as described above for the anti-TGF-β antibody inhibition experiment, except that glomerular cell cultures were used and RGD peptide was used to inhibit the accumulation of proteoglycan.

8. Page 3 of Exhibit B demonstrates the ability of platelet derived growth factor (PDGF) to inhibit the secretion of proteoglycans in a rat glomerular cell culture model. This experiment was designed essentially the same as described above for the anti-TGF-β antibody inhibition

experiment, except that glomerular cell cultures were used and PDGF was used to inhibit the accumulation of proteoglycan.

9. As further evidence of conception, provided as Exhibit C is a letter from Dr. Ruoslahti to Dr. Border mailed prior to December 22, 1988. In the letter Dr. Ruoslahti discusses results observed in experiments described in Exhibit B, pages 2 and 3, related to "the inhibition of TGFβ effects by PDGF" and "the inhibition of TGFβ effects by RGD." In addition to PDGF and RGD peptides as inhibitors of TGFβ, we had also contemplated, prior to December 22, 1988, anti-TGFβ antibody as another agent that could bind to TGFβ in a tissue and inhibit TGFβ effects to treat pathologies, as demonstrated by the Experiment related to Exhibit B, page 1.

10. As further evidence of conception of the claimed methods, and of diligence in reduction to practice, provided as Exhibit D is a copy of portions (cover page and pages 13 and 27) of a Grant Application executed and submitted on January 24, 1989. Section A.2.f. of page 13 of the Grant Application (Exhibit D), in the section related to a Research Plan-Specific Aim, in vivo, explicitly states that we contemplated "To develop regimens for therapeutic intervention in the disease model by antibodies and other agents capable of neutralizing the TGFβ effect." Paragraph "e." of page 27 of the Grant Application (Exhibit D), in the

section related to Experimental Design and Methods, explicitly states that:

> We have proposed several experiments that may provide agents that could block or ameliorate the action of TGFβ in the animal model of mesangial injury...It is conceivable that one or more of these agents could be administered to the animal and/or infused directly into the kidney as therapeutic agents to prevent the expansion of mesangial matrix...We expect that one or more of the agents to be tested will block the action of TGFβ. This information would be immediately applicable to the design of a study to treat humans with glomerulonephritis.

11. As further evidence of diligence in reducing to practice the claimed invention, we provide Exhibit E, which is copy of a portion (cover page, pages 2-5, 8 and 9) of an initial draft manuscript titled "An Antiserum Against Transforming Growth Factor β Suppresses Experimental Glomerulonephritis" as it existed in draft form on August 28, 1989. The draft manuscript summarizes results of numerous experiments conducted subsequent to the date of ordering the preparation of anti-TGFβ antibodies as set forth in Exhibit A, and subsequent the submission of the Grant Application on January 24, 1989, through the drafting of the manuscript on August 28, 1989. Pages 8 and 9 of Exhibit E describe the in vivo protocol corresponding to Example VII of the specification, used to to treat anti-Thy-1-induced nephritic

rats with control rabbit serum or anti-TGFβ serum. Pages 2-5 of Exhibit E describe the results of the experiments, in which it is specifically stated that:

> A surprise in our experiments was the involvement of TGFβ in acute glomerular disease and that the manifestations of this disease could be so effectively suppressed with anti-TGFβ treatment.

12. We do not know and do not believe that the invention was in the public prior to the time we conceived of the invention and reduced it to practice and we have never abandoned the application.

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false

statements may jeopardize the validity of the application or any patent issuing therefrom.

Respectfully submitted,

8-25-99
Date

Erkki Ruoslahti

Date

Wayne Border

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